Characteristics of microsatellite loci in Odonata

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Key words: Odonata, dragonfly, genome structure, genetic marker, microsatellites, molecular ecology.

Abstract

Microsatellite loci have become the genetic markers of choice for population-level molecular ecological studies. However, microsatellite loci had been isolated for comparatively few species of odonate until the past five years. This review summarises the main characteristics – expected heterozygosity and microsatellite length – of 116 microsatellite loci that have been isolated from the genomes of 11 odonate species and discusses potential problems associated with using microsatellite loci to study odonate biology. It is clear that odonates are characterised by relatively short microsatellites, typically less than 10 core motifs, that demonstrate a high level of heterozygote deficits. Some reasons why some odonate species have particularly low levels of gene diversity are discussed also.

Introduction

Genetic markers present an efficient, powerful, and flexible methodology to address fundamental ecological and evolutionary questions. For example, in natural populations it is now possible to accurately determine patterns of parentage and reproductive success, effective population size, demography, and to deconfound historic signatures of gene flow from contemporary patterns of dispersal (reviewed by Frankham et al. 2002; Excoffier & Heckel 2006; Selkoe & Toonen 2006). Of course, there are considerable differences in the characteristics of the suite of molecular-genetic markers that are available for these purposes, and it is crucial that the choice of marker is appropriate to the problem being tackled (Sunnucks 2000; Schlötterer 2004). When selecting a molecular marker one must be aware of different competing factors such as the level of polymorphism (i.e. mutation rate) of loci, their practical pitfalls, their functionality or neutrality and the time and expense associated with developing and using a particular technique. One of the most commonly-employed categories of genetic marker during the past decade is the microsatellite.

Microsatellite loci are tandem repeats of short (2-6 base pair) core DNA motifs, e.g. a pattern of two nucleotides 'CA' repeated ten times, and therefore they are also known as simple sequence repeats (SSR), variable number tandem repeats (VNTR), and short tandem repeats (STR) (Ellegren 2004). This class of genetic marker has

been found to be typically abundant in the genomes of many prokaryotic and eukaryotic organisms, occurring in both coding and non-coding regions. Different alleles at a microsatellite locus vary in the number of core repeat motifs, typically between five and 40 units, although longer arrays of repeat units appear typical of certain taxa, such as teleosts (Brooker et al. 1994; Amos 1999). Dinucleotide (2 base pair, bp, motifs), trinucleotide (3 bp motifs), and tetranucleotide (4 bp motifs) repeats are the most frequently employed categories of repeat unit for molecular genetic studies. Dinucleotide repeats would appear to account for the majority of microsatellites isolated for many species probably because they are the most abundant class of marker in the genome (e.g. Primmer et al. 1997; Fagerberg et al. 2001), although dinucleotide loci can suffer from problems when resolving alleles because of slippage during PCR that causes stutter banding. Trinucleotide and hexanucleotide (6 bp) repeats are the most likely repeat classes to appear in coding regions, and thus act as non-neutral loci, because they do not cause a frame shift when alleles expand or contract (Toth et al. 2000).

Mutation at microsatellite loci, which occurs in the form of slippage and proofreading errors during DNA replication, primarily changes the number of repeats (i.e. the length) of the microsatellite array (Ellegren 2004). Typically new alleles are formed by gain or loss of a single repeat motif, which is characterised by the stepwise model of mutation (SMM, Ohta & Kimura 1973). However, multi-step mutations, i.e. expansion or contraction of > 1 repeat motifs, have also been documented (Amos 1999; Xu et al. 2000; Harr et al. 2002; Huang et al. 2002). This type of mutation is addressed by the two-phase mutation model (TPM; Di Rienzo et al. 1994) that allows mutations of 1 repeat (one-phase) with probability p and mutations of ≥ 1 unit(s) (two-phase) with probability of $1 - p$. Irrespective of the appropriate mutational model, because alleles vary in length, rather than an alteration to the underlying sequence, they can be readily distinguished by acrylamide gel electrophoresis, thereby permitting high-throughput genotyping for a fraction of the cost and time compared with direct Sanger sequencing. Because most microsatellite loci are situated in non-coding genomic regions they can tolerate high rates of mutation, typically between 10^{-2} and 10^{-6} mutations per locus per generation, and on average 5×10^{-4}, which generates the high levels of polymorphism necessary for genetic studies of processes acting on ecological time scales (Schlötterer 2004).

For a variety of reasons, but most notably their high level of polymorphism, relative ease of scoring genotypes, co-dominance and abundance in many species' genomes, microsatellite loci have become the genetic "marker of choice" for studies of population genetic structure and parentage (reviewed by Sunnucks 2000; Schlötterer 2004; Selkoe & Toonen 2006). One potential drawback with using microsatellite loci, however, is that typically they must first be isolated de novo from the target species. Despite the considerable time required to isolate microsatellite loci this has not hindered panels of microsatellite loci being developed for thousands of species. Perhaps surprisingly, relatively few studies have isolated microsatellite loci from odonate genomes and used these loci for subsequent research; for example, a 'Web of Knowledge' search yields 20 hits for the keywords 'microsatellite' + 'Odonata', and 100, 180 and 390 hits for the terms 'microsatellite' + 'Lepidoptera', 'Diptera' and 'Hymenoptera' respectively. This paper summarises the main characteristics of presently published odonate microsatellite loci and discusses some of the issues that are associated with using microsatellite loci to study the evolutionary ecology of odonates.

Source of data

Panels of microsatellite loci have been published for relatively few species of Odonata, ordered with respect to their publication dates: *Ischnura elegans* (Vander Linden, 1820) (Cooper et al. 1996; n = 2 loci), *Megaloprepus coerulatus* (Drury, 1782) (Fincke & Hadrys 2001; Hadrys et al. 2005; n = 5 loci), *Coenagrion mercuriale* (Charpentier, 1840) (Watts et al. 2004b, c; n = 19 loci), *Erythromma viridulum* (Charpentier, 1840) (Keat et al. 2005; n = 10 loci), *Trithemis arteriosa* (Burmeister, 1839) (Giere & Hadrys 2006; n = 10 loci), *Anax imperator* Leach, 1815 (Hadrys et al. 2007a; n = 12 loci), *Orthetrum coerulescens* (Fabricius, 1798) (Hadrys et al. 2007b; n = 9 loci), *Coenagrion puella* (Linnaeus, 1758) (Lowe et al. 2007; n = 10 loci), *Anax junius* (Drury, 1773) (Matthews et al. 2007; n = 14 loci), *Ischnura hastata* (Say, 1839) (Lorenzo Carballa et al. 2007; n = 9 loci), and *Megalagrion xanthomelas* (Selys, 1876) (Jones et al. 2009; n = 16 loci). Therefore, the data summarised below are based on the characteristics of 116 loci isolated from 11 species of Odonata.

I extracted the following information for each microsatellite locus: (1) the longest number of uninterrupted repeat motifs that constitute the isolated microsatellite array; i.e. not the maximum number of repeats that could be inferred from the maximum reported allele size, (2) expected heterozygosity (H_e) and (3) observed heterozygosity (H_O). The longest contiguous number of repeats was used rather than dividing the loci into separate categories of pure and interrupted repeat motifs, as there are too few loci to make meaningful analyses of these categories. Expected heterozygosity was used to characterise levels of polymorphism rather than the number of alleles as it is less sensitive to the effect of the sample size used for genotyping.

Characteristics of microsatellite loci

The lengths of the (uninterrupted) microsatellite arrays isolated from odonate genomes vary considerably within and among species, but there is no obvious contrast between anisopterans and zygopterans. Over all species, the mean length of the microsatellite arrays varies from ca 7.5 repeat arrays in two species (*Erythromma viridulum* and *Trithemis arteriosa*) that also show only a modest amount of variation in array lengths among loci (i.e. small 95% confidence intervals), up to an average of ca 13 repeat units per locus in *Coenagrion puella*, which demonstrates a large amount of variation in array length among loci (Fig. 1).

Mean microsatellite array length is positively associated with expected heterozygosity (i.e. locus polymorphism), although this correlation is relatively weak (r = 0.27; Fig. 1); see also Figure 3 for the pattern of variation between array length and H_e for individual loci. In line with the interspecific variation in array length described above, there are considerable differences in the level of polymorphism between species (Fig. 1). All four species of anisopterans have average expected heterozygosities (H_e) greater than 0.6. By contrast, levels of genetic diversity vary widely between the species of zygopterans, with average H_e varying from a minimum of 0.39 in *E. viridulum*, to between 0.45-0.55 in *Coenagrion mercuriale*, *Ischnura elegans*, and *Megalagrion xanthomelas*, and achieving values greater than 0.70 in the remaining three species: *C. puella*, *Ischnura hastata*, and *Megaloprepus caerulatus*.

Heterozygote deficits are a common feature of odonate microsatellite loci, with 65% of loci (n = 74/113 loci for which data are available) having greater expected heterozygosities than observed heterozygosities (Fig. 2); of these loci, significant ($p < 0.05$) heterozygote deficits were reported in the original 'primer note' descriptions in 35 loci (~ 30% of loci). There is no obvious relationship between microsatellite array length (i.e. the number of uninterrupted repeats) and polymorphism (i.e. expected heterozygosity) and either of these two variables and whether or not heterozygote deficits are exhibited at a locus (Fig. 3). Moreover, the majority of loci clearly are rather short, with less than 10 contiguous repeat motifs identified in the original sequence descriptions (Fig. 3).

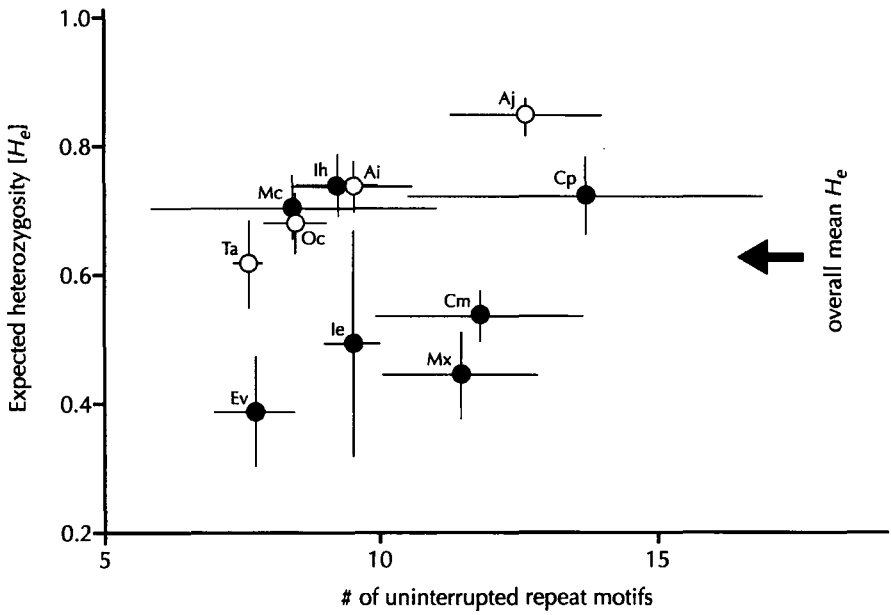

Figure 1: Microsatellite loci isolated from 11 odonate species — variation in mean (± 95% CI) number of uninterrupted repeat motifs against mean (± 95% CI) expected heterozygosity (H_e). Cm: *Coenagrion mercuriale* (Watts et al. 2004b, c; n = 19 loci); Cp: *C. puella* (Lowe et al. 2007; n = 10 loci); Ev: *Erythromma viridulum* (Keat et al. 2005; n = 10 loci); Ie: *Ischnura elegans* (Cooper et al. 1996; n = 2 loci); Ih: *I. hastata* (Lorenzo Carballa et al. 2007; n = 9 loci); Mx: *Megalagrion xanthomelas* (Jones et al. 2009; n = 16 loci); Mc: *Megaloprepus coerulatus* (Fincke & Hadrys 2001; Hadrys et al. 2005; n = 5 loci); Ai: *Anax imperator* (Hadrys et al. 2007a; n = 12 loci); Aj: *Anax junius* (Matthews et al. 2007; n=14 loci); Oc: *Orthetrum coerulescens* (Hadrys et al. 2007b; n = 9 loci); Ta: *Trithemis arteriosa* (Giere & Hadrys 2006; n = 10 loci). ●: Zygoptera; O: Anisoptera.

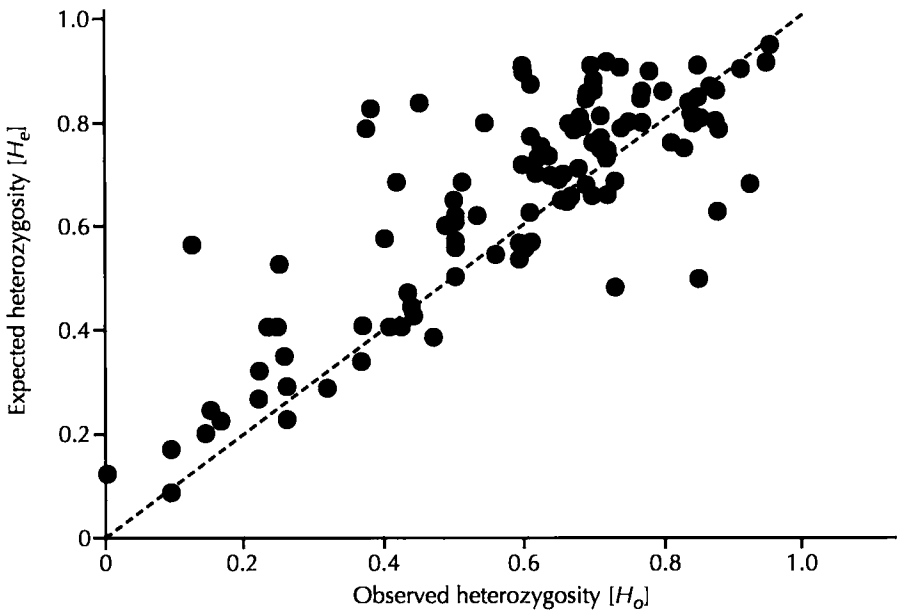

Figure 2: One hundred and thirteen microsatellite loci isolated from 11 species of Odonata — relationship between observed heterozygosity (H_O) and expected heterozygosity (H_e).

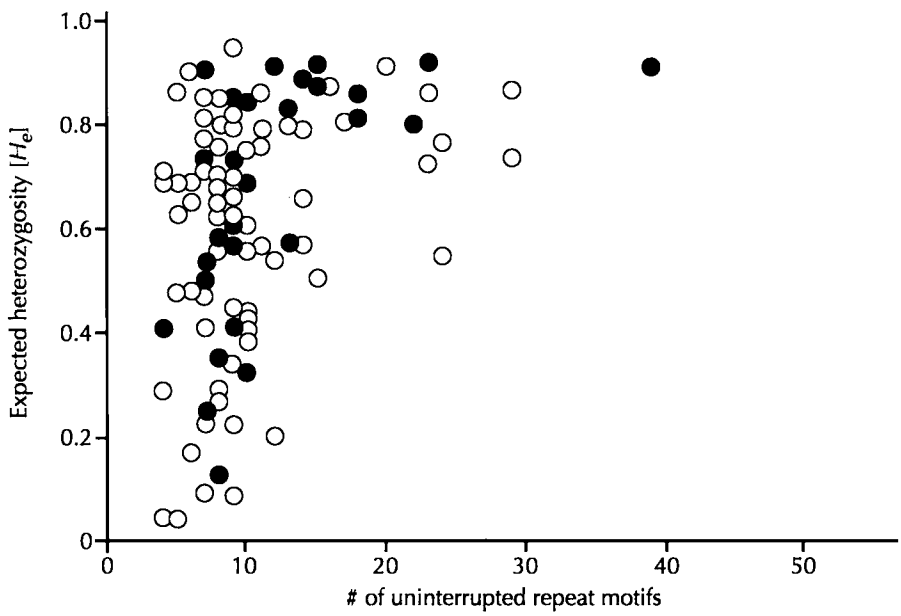

Figure 3: Individual locus variation in the number of uninterrupted repeat motifs against expected heterozygosity (H_e) at 113 microsatellite loci isolated from 11 odonate species. Coloured circles indicate loci with a reported significant ($p < 0.05$) heterozygote deficit.

Discussion

While several insect species, e.g. *Apis mellifera* (Estoup et al. 1993) and *Polistes annularis* (Hughes & Queller 1993), were among the first organisms to be studied using microsatellites, reasonable (> 5) numbers of microsatellite loci have been isolated and characterised from odonate genomes only during the last five years and still for just a handful of species. On the one hand this highlights a relative lack of contemporary genetic tools being used to determine patterns of dispersal and genetic diversity in odonate populations, and perhaps the lack of research into odonates compared with other insect taxa as highlighted in the Introduction. Of course, a number of research groups have used alternative genetic markers to address such issues; e.g. see Svensson et al. (2004) and Chaput-Bardy et al. (2008) who used amplified fragment length polymorphisms (AFLPs) to study populations of *Calopteryx splendens* (Harris, 1780). Alternatively, as the increasing interest in microsatellite loci coincides with the development of reliable enrichment protocols, the apparent paucity of microsatellite-based studies in odonate ecology may reflect technical difficulties associated with isolating and characterising microsatellite loci, some of which are highlighted by this analysis – for example, low abundance throughout the genome, lack of polymorphism, null alleles and/or failure to PCR-amplify across related species.

Compared with taxa such as mammals and teleosts, microsatellites are relatively scarce in insect genomes (Amos 1999; Fagerberg et al. 2001) and comparatively greater time and money is required to isolate large numbers of useable loci. Indeed, as mentioned in the introduction, far greater numbers of insect microsatellite-based studies have been directed towards economically and medically important insect taxa such as dipterans and hymenopterans. Unfortunately it is not possible to estimate the frequencies of microsatellite regions in a range of odonate genomes as most laboratories isolated their loci using an enrichment method. However, consistent with the idea that microsatellite loci occur infrequently in odonate genomes, is the observation that just nine microsatellite loci, defined as regions with seven of more repeat motifs arranged in tandem, were isolated from a partial genomic library (ca 25,000 cloned DNA fragments) constructed for *Ischnura elegans* (Cooper et al. 1996). Clearly it is not possible to determine the extent to which relative scarcity within the genome has limited the use of microsatellites to study odonates, however, it cannot have facilitated studies.

Another reason why few microsatellite loci have been isolated from odonates is that an uncommon motif may have been used to probe the partial genomic libraries. Typically, laboratories attempt to isolate $(CA)_n$ motifs most likely because it is the most abundant repeat motif in many model species' genomes (Tóth et al. 2000; Katti et al. 2001). There has yet to be a systematic screen of the most abundant motif in odonate genomes. However, insect genomes are typically AT-rich (Archak et al. 2006), as indeed are many plant genomes (Lagercrantz et al. 1993). While AT motifs apparently have not been used to probe odonate genomic libraries, both $(CA)_n$ and $(GA)_n$ probes were used to screen the genomic libraries of *I. elegans*, *I. hastata*, *Anax imperator*, *A. junius*, *Orthetrum coerulescens* and *Trithemis arteriosa*, yielding 23 and 33 microsatellites with predominantly CA and GA motifs respectively. It does not seem likely that there is a general trend for odonate genomes to favour GA repetitive core sequences more than CA, because there was considerable variation be-

tween species. For example, GA motifs were apparently most common in *A. junius* (Matthews et al. 2007), but the reverse was true for *T. arteriosa* (Giere & Hadrys 2006) and roughly equal numbers of repeat motifs were isolated from *A. imperator* (Hadrys et al. 2007a). Another apparent characteristic of the presently published odonate microsatellite loci is the predominance of dinucleotide microsatellite loci, compared with trinucleotide or indeed longer repeat motifs. This difference likely reflects the tendency to use dinucletide probes to screen genomic libraries, but is consistent with a generally lower frequency of trinucleotide (compared with dinucleotide) repeats in species' genomes (Primmer et al. 1997; Tóth et al. 2000; Katti et al. 2001; Prasad et al. 2005; but see also Archak et al. 2007); as researchers tend not to report whether other probes were used but failed to yield loci, it is not possible to differentiate between these two factors. Rather, this summary of published odonate microsatellite loci highlights that our knowledge of the characteristics, and concomitantly the distribution and function, of repetitive DNA in odonate genomes is meagre. A dot-blot analysis of odonate genomes would be a relatively simple method to quantify the relative frequencies of different microsatellite motifs and perhaps pave the way to develop insights into genome evolution in the odonates, and insects more generally.

In addition to low frequency of occurrence, a consistent feature of the published odonate microsatellite loci is that they typically contain 10 or fewer repeat motifs, with no obvious difference between Zygoptera and Anisoptera. Indeed, short loci appear to be typical of many insect microsatellite loci (Wilder et al. 2002; Archak et al. 2007), with Amos' (1999: fig. 6.1) review in particular highlighting that insects are characterised by microsatellites with ca 12 repeat motifs on average, compared with approximately 20 repeat motifs for fish and mammals. Variability at microsatellites is a function of mutation rate, and therefore, by implication it is possible that odonate microsatellites have relatively low mutation rates. Whatever the case, one implication of short microsatellites is generally low levels of variability, since microsatellite allele length broadly correlates with the amount of polymorphism (Schlötterer et al. 1998; Schug et al. 1998; Bachtrog et al. 2000). From a practical perspective this should guide researchers wishing to use microsatellite loci for odonate population genetics as, generally, greatest statistical power is achieved using markers that have intermediate levels of polymorphism. For example, very polymorphic loci are challenging to use because particularly large sample sizes are essential to get reliable estimates of allele frequencies. Equally, weakly polymorphic markers may present a lack resolution to detect population divergence, unless sufficiently many samples are collected to detect subtle differences in allele frequencies over sampling error. Despite this, there is a substantial degree of variability in level of polymorphism for loci of similar lengths (Fig. 3) that points to the clear influence of other factors in determining locus variability, such as, for example, locus-specific effects (see Harr et al. 1998) and recent demographic history.

Unfortunately, there are too few odonate microsatellite loci to allow an analysis of possible locus-specific effects upon genetic variability. Nonetheless, substantial variation in levels of gene diversity among species of odonates is indicative of some differences in genome structure or contrasting demographic histories. Again, a lack of more specific genomic data prevents any meaningful insights into possible variation in genome structure between these odonate species, although recent advan-ces in DNA sequencing technology (Mardis 2008) should make this type of research feasible.

For *Megalagrion xanthomelas,* the small genotyping sample size (n = 16 individuals for two sites in *M. xanthomelas*) is the most probably cause of the low level of expected heterozygosity, although further studies are required to confirm this as it is an island colonist. Analysis of gene diversity of *I. elegans* is complicated by the few (just two) loci that were developed (see e.g. the wide 95% CI on Fig. 1) and the presence of null (non-amplifying) alleles at one of these loci, at a relatively high frequency of ~ 0.39% (Cooper et al. 1996). Interestingly, contrasting demographic histories is likely to have generated the low levels of gene diversity in the remaining two species, *Coenagrion mercuriale* and *Erythomma viridulum*. Thus, samples of *E. viridulum* were collected from the edge of this species' range in the UK just a few generations after it colonised this area in 1999 (Dewick & Gerussi 2000), and only some 30 years after this species had increased its presence in north-western France (Ketelaar 2002). In this species, the lack of genetic diversity is presumably a consequence of population expansion via rapid colonisation of new habitats through a series of founder events (Keat 2007). By contrast, while *C. mercuriale* is also at its northern range margin in the UK, this species is presently experiencing a demographic decline in the UK due to loss and fragmentation of suitable habitat (Thompson et al. 2003). As such, many populations of this species are likely experiencing the effects of genetic erosion due to reduction in population size and subsequent failure to augment genetic diversity via immigration because dispersal is prevented among (increasingly) fragmented habitat patches (see Watts et al. 2004a, 2006; Thompson et al. 2007). Particularly striking is the extreme loss of diversity, with five of 14 microsatellite loci monomorphic, in a population of *C. mercuriale* that nonetheless persists at the extreme northwest edge of its range in Anglesey, North Wales, and is isolated from its nearest conspecifics by some 150 km (Watts et al. 2006; Thompson et al. 2007).

There are many reasons for heterozygote deficits, including unrecognised population subdivision (i.e. the Wahlund effect), assortative mating, non-random sampling of kin and section, and certainly these processes operate in odonate populations. Since the data on locus polymorphisms used here are based on genotypes of individuals from single source populations (a standard format for most primer notes) and significant ($p < 0.05$) heterozygote deficits were reported in every study, it is hard to invoke a mechanism whereby one or more of the biological effects mentioned above can account for the consistently large numbers of heterozygote deficits. Rather, it is more likely that there are technical problems associated with odonate microsatellite loci and a likely candidate for this is null alleles. Null alleles represent a major challenge to the use of microsatellites, particularly in studies of parentage. Null alleles are through to arise from mutation(s) in the sequence flanking microsatellite loci that affect primer binding during PCR. Certainly, nearly a third of the odonate loci appear to suffer from the presence of null alleles, and more generally there is a consistent trend for heterozygote deficits (Fig. 2). By implication, this would indicate a relatively high mutation rate in the genomic regions surrounding the microsatellite regions, which is surprising given the likely low mutation rate at the microsatellites themselves. Clearly, studies of parentage in odonates that use microsatellite loci must select the markers with care and particular attention should be given to the uses of appropriate software (e.g. Microchecker – van Oosterhout et al. 2003) to identify potential technical problems.

To date, studies that use microsatellites in odonates have determined patterns of sperm competition (Cooper et al. 1996) and parentage (Fincke & Hadrys 2001; Lowe et al. 2009), dispersal and spatial genetic structure (Watts et al. 2004a, 2006, 2007a), effective population size (Watts et al. 2007b), and putative hybridisation (Lowe et al. 2008). Given that cross-species amplification of microsatellite loci is possible in various taxa (e.g. Ellegren et al. 1997; Galbusera et al. 2000; Cunha & Watts 2007), including several species of Odonata (Watts et al. 2004b; Lowe et al. 2008; Jones et al. 2009), one might wonder whether genetic studies would be feasible for other odonate species without the need for further microsatellite development. One drawback of this approach is the characteristic reduction in the level of polymorphism observed in the non-target species (Ellegren et al. 1997; Galbusera et al. 2000; Watts et al. 2004b). For example, one study uncovered relatively poor-amplification success of loci developed for *C. mercuriale* in other members of the Coenagrionidae (Watts et al. 2004b). By contrast, data from Jones et al. (2009) indicate that a reasonable number (~ 69%) of loci will amplify in a congener, although the authors did not provide details about levels of null alleles or polymorphism. Thus, given the few loci isolated per species (typically ca 10) and the high proportion of null alleles in odonate microsatellites, it seems likely that a panel of microsatellite loci must be isolated specifically from the genome of the target odonate species de novo to provide sufficient polymorphic loci for meaningful statistical analyses.

Acknowledgements

I would like to thank Heike Hadrys, Oleg Kosterin, and Jessica Ware for their constructive comments on this manuscript.

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Received 10 March 2009; revised 28 May 2009; accepted 15 June 2009

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